

Certificate of Analysis

Flt1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-923, 14-923-K, 14-923M

Parent Lot # D12HP012N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal GST-tagged, recombinant, human Flt1 amino acids 783-end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione sepharose.

Purity 74% by SDS-PAGE and Coomassie blue staining. MW = 89kDa.

Specific Activity (Parent lot# D12HP012N): 344U/mg, where one unit of Flt1, active activity is defined as 1nmol phosphate incorporated into 250µM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.502mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

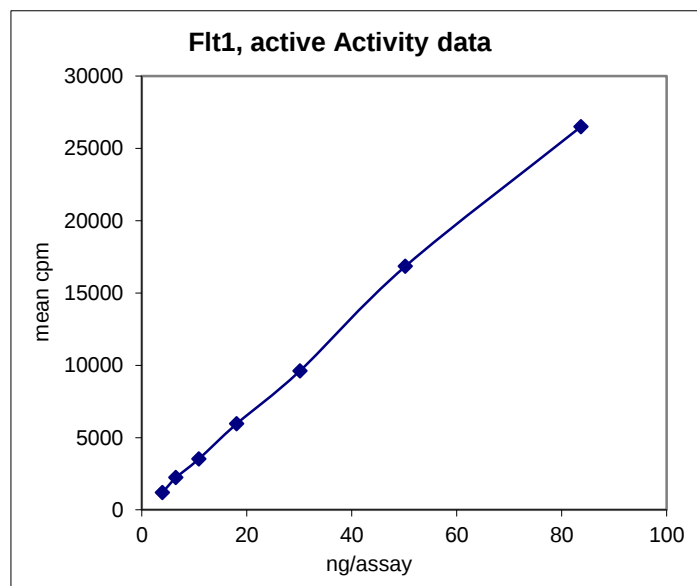
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

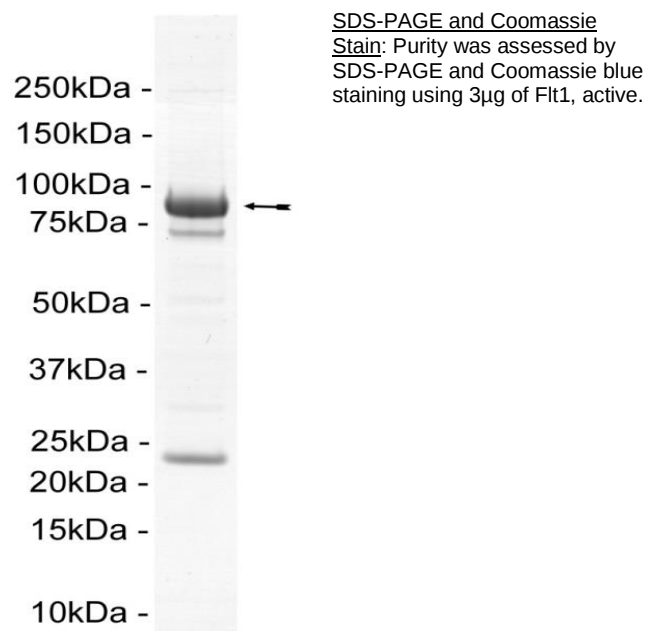
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4–84ng of this lot of enzyme phosphorylated 250µM (KKKSPGEYVNIEFG) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as Flt1 with the translated sequence listed on page four.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
3. **Flt1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4–84ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KKKSPGEYVNIEFG)**.
3. Add **2.5 μ l (4–84ng) Flt1, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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Flt1 Sequence Information

<u>Protein</u>	Human Flt1
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M227 of the recombinant protein is equivalent to M783 of human Flt1
<u>Accession number</u>	GenBank AF063657

Recombinant Flt1 amino acid sequence:

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1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSMa IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSMKRS SSEIKTDYLS
241 IIMDPDEVPL DEQCERLPYD ASKWEFARER LKLGKSLGRG AFGKVQASA FGIKKSPTCR
301 TVAVKMLKEG ATASEYKALM TELKILTHIG HHLNVVNLG ACTKQGGPLM VIVEYCKYGN
361 LSNYLKSKRD LFFLNKDAAL HMEPKKEKME PGLEQKKPR LDSVTSSSEF ASSGFQEDKS
421 LSDVEEEEDS DGFYKEPITM EDLISYSFQV ARGMEFLSSR KCIHRDLAAR NILLSENNIV
481 KICDFGLARD IYKNPDYVRK GDTRLPLKWM APESIFDKIY STKSDVWSYG VLLWEIFSLG
541 GSPYPGVQMD EDFCSRLREG MRMRAPEYST PEIYQIMLDC WHRDPKERPR FAELVEKLG
601 LLQANVQDGD KDYIPINAIL TGNSGFTYST PAFSEDFEKE SISAPKFNSG SSDDVRYVNA
661 FKFMSLERIK TFEELLPNAT SMFDDYQGDS STLLASPLMK RFTWTDSKPK ASLKIDLRVT
721 SKSKESGLSD VSRPSFCHSS CGHVSEGRKRR FTYDHAELER KIACCSPPPD YNSVVLYSTP
781  PI

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Recombinant Flt1 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tgcacttctt
61  ttggaatatt ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgtat
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1261 ttgagtgatg ttgaggaaga ggaggattct gacggtttct acaaggagcc catcactatg
1321 gaagatctga tttcttacag ttttcaagtg gccagaggca tggagttcct gtcttcaga
1381 aagtgcattc atcgggacct ggcagcgaga aacattcttt tatctgagaa caacgtggtg

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1441 aagatttgtg attttggcct tgcccgggat atttataaga accccgatta tgtgagaaaa
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1561 agcaccaaga gcgacgtgtg gtcttacgga gtattgctgt gggaaatctt ctccttaggt
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2161 agtaaaagta aggagtcggg gctgtctgat gtcagcaggc ccagtttctg ccattccagc
2221 tgtgggcacg tcagcgaagg caagcgcagg ttcacctacg accacgctga gctggaaagg
2281 aaaatcgcgt gctgctcccc gccccagac tacaactcgg tggtcctgta ctccaccca
2341 cccatctag

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